Methylcyclopentadienyl Manganese Tricarbonyl: Health Risk Uncertainties and Research Directions

J. Michael Davis

National Center for Environmental Assessment, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina

With the way cleared for increased use of the fuel additive methylcyclopentadienyl manganese tricarbonyl (MMT) in the United States, the issue of possible public health impacts associated with this additive has gained greater attention. In assessing potential health risks of particulate Mn emitted from the combustion of MMT in gasoline, the U.S. Environmental Protection Agency not only considered the qualitative types of toxic effects associated with inhaled Mn, but conducted extensive exposure–response analyses using various statistical approaches and also estimated population exposure distributions of particulate Mn based on data from an exposure study conducted in California when MMT was used in leaded gasoline. Because of limitations in available data and the need to make several assumptions and extrapolations, the resulting risk characterization had inherent uncertainties that made it impossible to estimate health risks in a definitive or quantitative manner. To support an improved health risk characterization, further investigation is needed in the areas of health effects, emission characterization, and exposure analysis. — *Environ Health Perspect* 106(Suppl 1):191–201 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-1/191-201Jdavis/abstract.html*

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Introduction

Methylcyclopentadienyl manganese tricarbonyl (MMT) is an organometallic compound developed by the Ethyl Corporation (Richmond, VA) in the 1950s to increase the octane level of gasoline and thereby enhance the antiknock properties of the fuel. It also was marketed to improve the combustion of fuel oil and turbine fuel (*1*). Even before the introduction of MMT into the U.S. fuel supply began in 1976, concerns were raised about its potential public health implications (*2*). After passage of the 1977 amendments to the Clean Air Act (CAA) (*3*), however, the manganese (Mn) additive was legal for use only in leaded gasoline. According to the Ethyl Corporation (*4*) over 70 million pounds of MMT have been sold for use in U.S. leaded gasoline since 1976. In 1979, while crude oil was in short supply, the U.S. Environmental Protection Agency (U.S. EPA) also allowed MMT to be added to unleaded gasoline for a few months. The limited usage of MMT in leaded gasoline was permitted until lead was phased out of U.S. gasoline at the end of 1995.

The Ethyl Corporation has applied to the U.S. EPA several times since 1978 for permission to sell MMT for use in unleaded gasoline. Section 211(f) of the CAA (*3*) makes it unlawful for a manufacturer to introduce any fuel or fuel additive (F/FA) that is not substantially similar to F/FAs used in the certification of 1975 or later vehicle engines. This prohibition against new F/FAs may be waived if an applicant establishes that the F/FA will not cause or contribute to an exceedance of vehicle emission standards. If the U.S. EPA Administrator does not act to grant or deny a waiver petition within 180 days of receipt of the application, the statute provides that the waiver shall be automatically granted. Four waiver petitions submitted by the Ethyl Corporation were denied because of concerns regarding increases in exhaust hydrocarbon emissions resulting from MMT use. During consideration of the third and fourth applications, the U.S. EPA also raised concerns regarding the possible adverse health effects of an increase in airborne Mn resulting from MMT use. Ultimately, in July 1994, the U.S. EPA Administrator denied the Ethyl Corporation's waiver petition specifically because of concerns about risks to public health (*5*). In 1995, the Ethyl Corporation successfully challenged the denial of its petition in federal court (*6*). The court ruled that Section 211(f)(4) of the CAA (*3*) provides no basis for U.S. EPA to deny Ethyl's petition on any grounds except whether MMT would cause or contribute to a failure of any emission control device or system.

In May 1994, as mandated in CAA (*3*) Section 211(b) and (e), the U.S. EPA issued the F/FA rule (*7*), which required that F/FA manufacturers provide evidence from existing studies or conduct new tests to address specified health end points (i.e., at a minimum, subchronic inhalation toxicity studies that include reproductive and developmental toxicity, neurotoxicity, and mutagenicity end points). Under the F/FA rule, manufacturers whose products were not chemically similar to F/FAs previously registered by the U.S. EPA could not market their products until specified toxicity and other information was provided to the agency. Although the U.S. EPA maintained that MMT had not been previously registered, the Ethyl Corporation successfully challenged that position as well (*8*). Consequently, the Ethyl Corporation has been marketing MMT to U.S. oil refineries since December 1995. However, as provided by the F/FA rule, studies prescribed

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Address correspondence to Dr. J.M. Davis, National Center for Environmental Assessment, U.S. Environmental Protection Agency (MD-52), Research Triangle Park, NC 27709. Telephone: (919) 541-4162. Fax: (919) 541-0245. E-mail: davis.jmichael@epamail.epa.gov

Abbreviations used: CAA, Clean Air Act; CNS, central nervous system; F/FA, fuel and fuel additive; IRIS, integrated risk information system; LOAEL, lowest observed adverse effect level; MMT, methylcyclopentadienyl manganese tricarbonyl; NOAEL, no observed adverse effect level; PM, particulate matter (subscript number indicates fiber particle size in μm); PTEAM, particle total exposure assessment methodology; RfC, inhalation reference concentration; RfD, oral reference dose; TEL, tetraethyl lead; TLV, threshold limit value; U.S. EPA, U.S. Environmental Protection Agency; VMT, vehicle miles traveled.

by the U.S. EPA must still be conducted within a specified time frame even though MMT is already in use.

The Canadian government has permitted the use of MMT in gasoline in all provinces since 1978 but recently banned the importation of MMT, which could effectively eliminate its use in Canadian gasoline. The Ethyl Corporation indicated that it would challenge the Canadian importation ban under provisions of the North America Free Trade Agreement (*9*). Although MMT is apparently permitted in Argentina, Australia, Russia, and conditionally in New Zealand (*10*), whether it is actually used in any countries apart from the United States and Canada is unknown.

Because MMT is relatively inexpensive as an octane enhancer, it could be in fairly widespread use across the United States. However, the 1990 CAA Amendments (*11*) specifically prohibit the use of fuel additives containing metals in areas designated for the use of reformulated gasoline. Except for such areas (including the entire state of California, which specifically banned MMT in 1991), MMT is legal for use in most of the gasoline sold in the United States. Moreover, other provisions of the 1990 CAA Amendments (*11*) allow the Ethyl Corporation to petition separately for a waiver to market MMT even in reformulated gasoline areas. Although several major petroleum companies have indicated that they would not add MMT to their products, a large market may still exist in the United States.

In formulating policy on the use of MMT, particular caution has been exercised because of previous experience with adverse public health impacts associated with extensive population exposure to a heavy metal from a fuel additive in gasoline, namely tetraethyl lead (TEL) (*12*). Blood lead levels in the U.S. population correlate with changes over time in the use of TEL in gasoline, a fuel used pervasively in the United States. This correlation is compelling evidence of how a relatively small concentration of a fuel additive may result in significant exposure of the general population (*13*). In the case of TEL in gasoline, the primary exposure issue was the combustion emissions of particulate lead from vehicles, rather than the fuel additive itself. As lead was emitted from tail pipes and dispersed into the atmosphere, population exposures occurred primarily by inhalation of particles in the ambient air and secondarily from ingestion of these particles deposited on food, soil, and other surfaces with which the entire population or particular subpopulations (e.g., young children) came into contact. As with TEL the U.S. EPA assessment of the potential health risks associated with MMT was concerned primarily with combustion emissions from vehicles, not the fuel additive itself. Given that the allowable concentration of MMT in U.S. fuel is only 1/32 (0.031) g Mn/gal of gasoline and that the compound undergoes rapid photodegradation in the atmosphere (*14*), MMT itself was not considered a significant exposure risk for the general population, except for accidental or occupational contacts.

Unlike lead, the oral route of exposure is considered less of a threat with Mn. Dietary Mn has relatively low toxicity at most exposure levels due in part to a low rate of absorption from the gastrointestinal tract and in part to efficient regulation by homeostatic mechanisms (*15*). The Mn oral reference dose (RfD) is 0.14 mg/kg/day for dietary sources. However, for nondietary sources such as water or soil and especially for infants consuming formula prepared with water, a value of 0.05 mg/kg/day is specified by the U.S. EPA (*15*). The Mn RfD reflects the fact that Mn is considered a nutritionally essential trace element; it is required for enzymes such as hydrolases, kinases, decarboxylases, and transferases, and for metalloenzymes such as mitochondrial superoxide dismutase, which are important for normal functioning of the central nervous system (CNS) and other systems. However, Mn toxicity to workers exposed by inhalation has been recognized since the early 1800s (*16*). Unlike ingested Mn, inhaled Mn is transported directly from the respiratory tract to the brain before its first pass by the liver. Depending on the form of Mn inhaled, its conversion to other oxidation states (e.g., oxidation of Mn^{2+} to Mn^{3+} or reduction of Mn^{4+} to Mn^{3+}), and its ability to enter the brain through a protein transport mechanism or otherwise (*17–19*), a significant fraction of inhaled Mn may reach target sites in the CNS, especially the basal ganglia. Some evidence indicates that uptake may occur via the nasal mucosa, with direct olfactory axonal transport to the CNS (*20*), as well as via alveolar absorption into the bloodstream.

According to an early report from the Ethyl Corporation (*21*), the combustion of MMT in gasoline produces submicron particles of Mn tetraoxide (Mn_3O_4), which contains both Mn^{2+} and Mn^{3+}. More recent analyses provided to the U.S. EPA by the Ethyl Corporation indicate the presence of a Mn phosphate compound containing only Mn^{2+}. At this writing, the form or forms of Mn produced by combustion of MMT in gasoline have not been definitively ascertained.

U.S. EPA Health Risk Assessment of MMT

The evaluation of the potential health risks associated with the use of MMT in unleaded gasoline followed the National Research Council paradigm of identifying the types of health hazards, characterizing the exposure–response relationship, and relating this information to a human exposure assessment (*22*). Because very little information was available on the inhalation toxicity of Mn_3O_4, thought to be the primary combustion product of MMT in gasoline, the U.S. EPA assessment of MMT (*23*) considered inhalation data on any form of Mn. Most of this information came from studies of occupational populations and from some laboratory animal experiments. Primary features of excessive occupational exposure to Mn include neurobehavioral, respiratory, and reproductive effects. Manganism is characterized by various psychiatric and movement disorders and usually consists of two or three phases (*24*). The first is a psychiatric aspect that includes disturbances such as excessive weeping and laughing, sleep disturbances, irritability, apathy, and anorexia. The term manganese madness (*locura manganica*) is sometimes applied to these symptoms of Mn toxicity (*25*). A second phase consists of neurologic and neuromuscular signs such as gait disturbances, dysarthria, clumsiness, muscle cramps, intention tremor, and masklike facial expressions. In addition a final stage of Mn intoxication may involve symptoms of irreversible dystonia and hyperflexion of muscles that may not appear until many years after the onset of exposure (*25*). Similarities between the later stages of manganism and Parkinson's disease have been noted frequently because of the impairment of fine motor control, lack of facial expression, and apparent involvement of underlying common neuroanatomical and neurochemical factors (*26,27*). However, most clinical neurologists now differentiate, both functionally and neuroanatomically, between the two afflictions of the extrapyramidal tracts (*28*).

The precise mechanisms of Mn neurotoxicity are not well understood, but it appears that Mn can affect several different aspects of CNS structure and function.

Postmortem examinations of Mn-exposed humans and experimental studies of Mn-exposed laboratory animals indicate that Mn is distributed preferentially to nuclei of the basal ganglia, particularly the caudate, putamen, globus pallidus, subthalamic nucleus, and substantia nigra, and to a lesser extent to other regions of the brain, including the cerebellum and pituitary (*29–31*). Generally the primary sites of neuropathologic changes associated with Mn toxicity in humans and experimental animals are the basal ganglia, particularly the globus pallidus, caudate, and putamen. There is little or no involvement of the substantia nigra (*29–33*). Investigation of the mechanisms of Mn neurotoxicity has focused on the oxidative properties of Mn (especially Mn^{3+}) and its interactions with the dopaminergic system, including biphasic increases and decreases in dopamine levels associated with Mn exposure (*34*). The role of nigrostriatal pathways and dopaminergic and γ-aminobutyric neurons in Mn neurotoxicity has not been fully established; some evidence suggests that the nigrostriatal pathway itself remains intact, with damage occurring postsynaptically (*31,35*). Mitochondrial uptake and disruption have also been investigated as mechanisms of Mn neurotoxicity (*36,37*).

Other organs and systems are also targets of Mn toxicity. Respiratory effects such as pneumonia, susceptibility to acute infections of the respiratory tract, and cough have been noted in Mn workers (*38,39*). One study has suggested a possible increase in prevalence of respiratory illnesses in school children residing near a point source of atmospheric Mn pollution (*40*). Occupational case reports frequently indicate that reproductive function may be impaired by Mn exposure, often manifested by symptoms such as loss of libido, impotence, and similar complaints (*24,41*). Lauwerys et al. (*42*) reported a statistically significant decrease in the number of children born to workers exposed to Mn dust during the ages of 16 to 25 and 26 to 35 years, as compared to control workers. Disturbance of the hypothalamic–pituitary–gonadal axis hormones has been implicated in Mn toxicity by other investigators (*43*).

In addressing carcinogenic effects the U.S. EPA placed Mn in a Group D weight-of-evidence category, which signified that it was not classifiable as to human carcinogenicity because of mixed or insufficient evidence (*44*). Given this fact, together with the more extensive database on the neurobehavioral effects of Mn and some indications that respiratory and reproductive systems may not be as sensitive to Mn exposure as is the CNS (*45*), the U.S. EPA health assessment focused on the potential for chronic noncancer effects, particularly neurotoxic effects.

The exposure–response aspect of the assessment consisted of the inhalation reference concentration (RfC) for Mn. The RfC is an estimate (with uncertainty spanning about one order of magnitude) of a continuous inhalation exposure level for the human population (including sensitive subpopulations) that is likely to be without appreciable risk of deleterious noncancer effects during a lifetime. In 1990 the U.S. EPA issued an RfC for Mn of 0.4 $\mu g/m^3$ (*46*). As additional information and published studies became available in the course of reconsidering the waiver petition for MMT, the RfC was revised in 1993 (*47*) and incorporated into a reevaluation of the potential inhalation health risks associated with MMT (*23*).

Inhalation Reference Concentration for Manganese

The 1993 RfC for Mn (*47*) was based primarily on a study of Mn-exposed alkaline-battery plant workers in Belgium by Roels et al. (*45*). This cross-sectional study evaluated neurobehavioral and other end points in 92 male workers exposed to Mn dioxide (MnO_2) dust, compared to a matched control group of 101 male workers without industrial Mn exposure. The geometric mean occupational-lifetime integrated respirable fraction of particulate matter (PM_5) (subscript number indicates particle size in μm) dust concentration was 793 $\mu g\ Mn/m^3 \times$ years (range: 40–4,433). The Mn-exposed workers performed significantly worse than matched controls on several measures of neurobehavioral function, particularly tests of eye–hand coordination, hand steadiness, and visual reaction time.

Similar neurobehavioral impairments were also found in an earlier study by Roels et al. (*39*) of a different occupational population in Belgium exposed to comparable (total dust) concentrations of mixed Mn oxides and salts. In addition, a study of Canadian workers by Mergler et al. (*48*) indicated that compared to matched controls, performances on tests of rapid alternating hand movements, hand steadiness, and other aspects of fine motor control were significantly worse in workers who had been exposed to even lower concentrations of respirable Mn dust than those measured by Roels et al. (*45*). However, information on past exposure levels was not provided by Mergler et al. (*48*). Reports of a Swedish study of Mn-exposed steel workers (*49*) also have provided evidence of comparable neurobehavioral impairments, including slower reaction time and finger-tapping speed. Collectively these studies, now complemented by more recent work (*50*), constitute compelling evidence of neurotoxicity associated with low-level occupational Mn exposure below the then-current threshold limit value (TLV) of 5 mg/m^3. [The American Conference of Governmental Industrial Hygienists (*51*) has since revised the TLV to 0.2 mg/m^3.] The fact that the speed and coordination of motor function are especially impaired is noteworthy because of the consistency with the other epidemiologic, clinical, and experimental animal evidence of neurotoxicity at higher concentrations of Mn (*47*).

In deriving an RfC for Mn, the geometric mean integrated respirable dust concentration (793 $\mu g\ Mn/m^3 \times$ years) from Roels et al. (*45*) was divided by the average period of worker exposure (5.3 years) to eliminate time (in years) from the time-weighted average, thereby yielding a lowest observed adverse effect level (LOAEL) of 150 $\mu g\ Mn/m^3$. Other adjustments made to relate the occupational exposure (e.g., 5 days/10m^3 air breathed per week, day) to lifetime environmental exposure (7 days/20m^3 air breathed per day) produced an exposure-adjusted LOAEL of 50 $\mu g\ Mn/m^3$. This value was then divided by a total uncertainty factor of 1000 to yield an RfC of 0.05 $\mu g/m^3$. The total uncertainty factor of 1000 incorporated the following factors: 10 to protect sensitive individuals (e.g., infants, pregnant women, elderly), 10 for using an LOAEL in lieu of a no observed adverse effect level (NOAEL), and a composite factor of 10 for database limitations. The composite factor subsumed three areas of uncertainty: extrapolation from subchronic to chronic exposure, inadequate reproductive and developmental data, and unknown differences in the toxicity of different forms of Mn.

After the U.S. EPA issued the RfC for Mn in 1993 (*47*), the Ethyl Corporation requested that the U.S. EPA consider alternative approaches to analyzing the data of Roels et al. (*45*) and including supplementary data provided by Roels (*52*). One objective of such analyses was to identify an NOAEL and thereby eliminate or reduce one uncertainty factor of 10 in the

derivation of the RfC. In response U.S. EPA scientists applied a variety of statistical approaches proposed by Ethyl or under consideration by the U.S. EPA, including benchmark dose analyses of the type described by Crump (*53*) and Bayesian analyses of the type described by Hasselblad and Jarabek (*54*). With several choices of exposure measures, effects measures, exposure–response models, and uncertainty factors, more than 100 RfC estimates were obtained by applying various statistical methods. Of these the most plausible estimates for alternatives to the Mn RfC of 0.05 μg/m^3 ranged from approximately 0.09 to 0.2 μg/m^3 (*23,55*).

Exposure Assessment

Although ambient air levels may provide a first step in attempting to assess human exposure to particulate Mn, personal exposure levels of automotive-source pollutants may be uncorrelated with or underestimated by ambient air levels (*56*). Data on personal exposure levels of Mn likely to be associated with the use of MMT as an additive in unleaded gasoline have been limited. Some studies (*57,58*) of small samples of taxi drivers, garage workers, and office workers/commuters were conducted in Canada, where the allowable MMT concentration in gasoline was 1/16 (0.062) g Mn/gal. [Although as much as 1/16 g Mn/gal was permitted in Canada, the concentration actually used was probably closer to the U.S. limit of 1/32 (0.031) g Mn/gal (*59*).] These studies had inadequate sample sizes and lacked probabilistic statistical designs that would help ensure the representativeness of the sampled individuals. Moreover, meteorologic and other factors that might have affected ambient air Mn measurements over the relatively short time periods of these studies (1 to 2 weeks at most) were not adequately characterized. Because of limitations such as these, no quantitative assessment of personal exposures to Mn in a Canadian population was possible. However, some studies (*60*) have clearly indicated a general relationship between personal exposure levels of Mn and proximity to vehicular emissions of combusted MMT. Thus populations living near high traffic-volume areas, for example, would be expected to experience higher Mn exposure levels (*61*).

The only published study that has used a probability-based representative sampling design for evaluating exposure levels of Mn in a general population was the particle total exposure assessment methodology (PTEAM) study (*62*). Although this study was not conducted with a particular focus on either Mn or MMT, it provided valuable information on potential Mn exposure associated with MMT use because MMT was an additive in leaded gasoline in California prior to and during the period of the PTEAM study. The study was conducted in Riverside, California, over a 7-week period in the fall of 1990 and used personal and stationary monitors to measure Mn concentrations indoors and outdoors. Of the 139,000 nonsmoking residents 10 years of age and older in Riverside, 178 individuals were selected through a stratified sampling plan to represent the general population and were monitored over two 12-hr periods (daytime and nighttime). More than 2750 air samples of particles were collected.

In the PTEAM study, measurements of personal exposure levels of PM_{10} Mn indicated that approximately half of the population in Riverside in the study period had 24-hr personal exposures to PM_{10} Mn exceeding 0.035 μg/m^3, with the highest 1% of the population having exposures above 0.22 μg/m^3 PM_{10} Mn. However, because the RfC for Mn was based on PM_5 dust measurements in the study of Roels et al. (*45*), it was preferable to consider a population distribution of personal exposure levels of PM_5 Mn to more closely relate exposure and effect metrics. In addition it was necessary to project from the situation in Riverside around the time of the PTEAM study (when leaded MMT gasoline constituted about 14% of the gasoline sold and contained an average of 0.048 g Mn/gal) to a future scenario that assumed 100% of the unleaded gasoline would contain MMT at 1/32 (0.031) g Mn/gal.

The derivation of the projected exposure estimates involved several steps and extrapolations (*23*) to yield two projections of long-term personal exposure levels of PM_4 Mn (the nearest approximation possible to PM_5) in relation to MMT usage at 1/32 g Mn/gal in 100% of unleaded gasoline. Albeit long term, these projections were limited to the fall season and could not be considered estimates of annual average exposure levels. Two estimates are presented because alternative approaches were employed to relate $PM_{2.5}$ measurements from stationary indoor monitors to PM_4 personal exposure levels. In essence it was estimated that half of the population could experience PM_4 Mn exposure levels of greater than approximately 0.045 to 0.050 μg/m^3. Also, based on the two projection estimates, approximately 5 to 10% of the population could have personal exposure levels around 0.1 μg/m^3 PM_4 Mn or higher, with the highest 1% above 0.15 μg/m^3.

Note that these projections refer specifically to Riverside, California, but are probably reasonably representative of the greater Los Angeles metropolitan area (population > 14.5 million). Although the Riverside-based estimates cannot be applied quantitatively to any other U.S. metropolitan areas, the projections may have some relevance to other U.S. cities or locales (e.g., in the Southwest) that qualitatively resemble Riverside in meteorology, traffic patterns, and other characteristics of relevance to automotive Mn levels. The presence of a major point source or sources of Mn in a community (not a factor in Riverside) would also add some increment to the level of Mn exposure experienced by persons in that community. Possibly, then, several hundreds of thousands of individuals could be exposed to PM_4 Mn levels of approximately 0.1 μg/m^3 or higher if MMT were used in 100% of the unleaded gasoline at 1/32 g Mn/gal in all of these areas. However, it must be emphasized that because of the limited available exposure data, a great deal of uncertainty surrounds such estimates. The actual exposure levels could be much higher or lower.

Risk Characterization

To assess health risks, qualitative and quantitative health effects information must be related to available exposure information. From the standpoint of a qualitative hazard identification, the available evidence amply demonstrates that inhaled Mn is toxic to the nervous system, the respiratory system, and the male reproductive system. Although occupational epidemiologic findings of impaired motor function (e.g., reductions in eye–hand coordination, slower hand or finger movements, and less control of fine movement) may be subtle and probably not readily evident to clinical physicians, they are nevertheless significant from a public health standpoint when considered as potential population effects.

Quantitative dose–response analyses of such neurobehavioral data support a range of approximately 0.09 to 0.2 μg/m^3 as possible alternatives to the value of 0.05 μg/m^3 that was established as the Mn RfC in 1993 and remains as such on the U.S. EPA Integrated Risk Information System database (*47*). By definition RfC analyses do

not yield a precise concentration that defines a demarcation between safety and hazard. Rather, interpretation of a Mn RfC estimate is best made in relation to an assessment of population exposures to Mn, with the understanding that the RfC is a protective level, not a predictive value.

The exposure assessment provides reasonable but necessarily uncertain estimates of personal exposure levels of Mn that might result from the use of MMT in gasoline. These estimates indicate that if MMT (at 1/32 g Mn/gal) were used in all unleaded gasoline in Riverside, California, approximately 40 to 50% of the population could experience PM_4 Mn exposures exceeding the 1993 RfC of 0.05 $\mu g/m^3$ (derived from PM_5 Mn health effects data) and approximately 5 to 10% could experience PM_4 Mn exposure levels around 0.1 $\mu g/m^3$ or higher.

Figure 1 juxtaposes projected personal exposure distributions and selected RfC values. It is evident that some candidate RfC estimates as well as the 1993 verified RfC are in the range of the exposure estimates. Exceeding the RfC does not necessarily indicate that a public health risk exists. With orders of magnitude separating an LOAEL or NOAEL from the RfC, it is impossible to state whether projected exposures above the RfC would lie above or below a presumed threshold level for adverse health effects on the actual concentration–response curve for Mn neurotoxicity. Obviously, however, this inability to state definitively that adverse health effects will occur cannot be interpreted to imply that no health risk exists at exposure levels exceeding the RfC. In short, although it is not possible to quantify an estimation of risk, a reasonable basis exists for concern regarding potential public health risks, especially for susceptible subpopulations, if MMT is used widely in unleaded gasoline. For a more definitive estimate of risk, it is essential that more information be obtained on exposure to and health effects of Mn combustion products of MMT in gasoline.

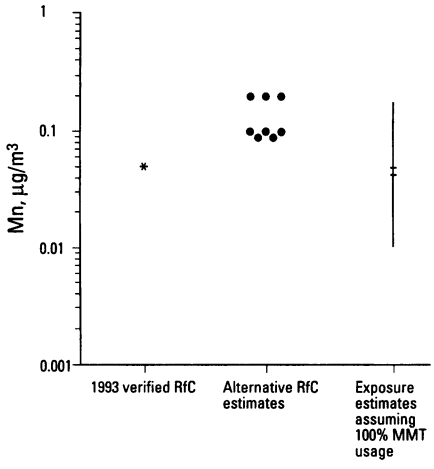

Figure 1. Comparison of Mn RfC values and projected distributions of personal exposure levels of Mn. Two exposure distributions are collapsed into a single vertical line, with the median of each distribution represented by a horizontal mark.

Uncertainties

Uncertainties are inherent in any risk assessment. In the case of the MMT assessment, both the exposure–response analyses and the exposure assessment contain assumptions, extrapolations, and judgments, each of which introduces uncertainties to the risk characterization. In the RfC analyses uncertainties are formally represented by numerical uncertainty factors, as well as by the fact that, by definition, an RfC reflects uncertainty spanning perhaps an order of magnitude. RfCs are designed to be more protective (i.e., lower) when there is a greater number or degree of uncertainties or information gaps.

These uncertainties require elaboration if they are to be appreciated as more than semiquantitative representations of concerns. An uncertainty factor of 10 for protection of sensitive individuals is commonly applied in deriving RfCs, particularly from epidemiologic data limited to healthy adult workers. Susceptible subpopulations may have an increased potential for excessive Mn body burdens attributable to increased absorption or altered clearance mechanisms, as suggested by limited data on children (*63,64*), pregnant women (*65,66*), elderly persons (*67*), iron- or calcium-deficient individuals (*18,68,69*), and individuals with liver impairment (*70,71*). Children are of special concern for several reasons. As the long-term or irreversible effects of exposure to lead and other developmental neurotoxicants during certain critical stages of development have illustrated (*72*), Mn exposure during a limited period of early development might be capable of inducing permanent or irreversible damage to the developing CNS. Recent work comparing the neurotoxic effects of Mn in rats at different ages (*73,74*) supports a concern about greater sensitivity to Mn neurotoxicity during early development. Moreover, several studies (*43,75–77*) have demonstrated alterations in dopamine levels in young mice and rats exposed to Mn via noninhalation routes during early postnatal development. Such findings raise concerns about the potential for adverse impacts on children. Young children could also be at higher risk because of metabolic differences, as suggested by the fact that homeostatic mechanisms for regulating Mn absorption and elimination are not well developed in infants (*63,78,79*).

The elderly are another special population of concern. At least one report of subchronic Mn poisoning in humans via the oral route points to a greater vulnerability of the elderly (*67*). Also, over time Mn body burdens and/or small impairments in neurobehavioral function may accumulate. If neurobehavioral function is already compromised by normal aging processes or disease states (e.g., preclinical Parkinsonism), the ability to compensate for declines in neurobehavioral function could be eventually overwhelmed by additional, albeit possibly quite small, insults attributable to Mn (*80,81*). Note that Parkinson's disease is typically a geriatric disease in which symptoms are seen only when the loss of brain cells that produce dopamine (the loss of which may also be involved in Mn toxicity) reaches 80% or more. If Mn were to contribute to such losses, the effect could be a more severe or earlier onset of declining function in senescence (*82,83*).

Recent epidemiologic studies of occupational cohorts do little to reduce this concern because of the relatively young populations studied. The oldest worker in the study by Roels et al. (*45*) was less than 50 years of age and the average age in that study was only 31.3 years. Average worker ages in other key studies were also low: 34.3 (*39*), 43.4 (*48*), and 46.4 (*49*) years of age. If the elderly actually are more susceptible to even subtle manifestations of Mn toxicity, the implications for reduced function and consequent increases in societal healthcare costs could be enormous.

Individual differences in susceptibility to Mn toxicity have long been apparent in the occupational medicine literature (*68,84*). The U.S. EPA assessment of MMT noted that individual data for two workers in the study by Roels et al. (*45*) suggested that they may have been especially susceptible to the neurobehavioral effects of occupational Mn exposure as reflected by their abnormal scores and limited exposure durations. Attempts to fit an exposure–response model to the Roels (*52*) data with these two subjects included resulted in a supralinear curve (*55*). One of the difficulties in investigating such individual differences in worker populations is that inherently

susceptible individuals would probably be more likely to change to other types of employment, which leaves a greater proportion of relatively less sensitive individuals among the Mn worker population.

Another concern identified in deriving the Mn RfC included the lack of chronic exposure data. In the 1992 Belgian study (*45*) the mean period of exposure was only 5.3 years (range: 0.2 to 17.7 years), with a marginally longer exposure of 7.1 years in the earlier Belgian study (*39*). In the Swedish (*49*) and Canadian (*48*) studies the mean durations of exposure were somewhat longer: 9.9 and 16.7 years. It is quite possible that longer exposure periods or testing of older workers might result in the detection of effects at lower concentrations than is possible after shorter periods of exposure or in younger workers. On the other hand it is also evident from these studies that a relatively short period of occupational Mn exposure may be sufficient to induce Mn neurotoxicity. A related concern is that some manifestations of Mn toxicity may become evident only after a considerable lag, possibly several years after exposure occurred or terminated (*24,25*).

An uncertainty factor for extrapolation from a subchronic exposure to chronic exposure was included in deriving the Mn RfC estimates. However, a half factor of only 3 (on a base 10 logarithmic scale) was used for this area of uncertainty. If the average period of Mn exposure (4 years geometric mean) in the Roels et al. study (*45*) is compared to an assumed lifetime of 70 years, one could argue that a factor of 70/4 = 17.5, or at least 10, would be a more appropriate adjustment for subchronic to chronic exposures. In general the U.S. EPA has used an uncertainty factor of 3 for subchronic-to-chronic extrapolation for other chemicals with comparable databases. It may be fair to say, however, that in this instance a factor of 3 probably did not err in the direction of being too conservative (protective).

Also reflected in the composite uncertainty factor of 10 is the lack of adequate data on reproductive and developmental effects. No known studies have investigated human female reproductive function, and male worker reproductive function has not been extensively investigated even though it is affected by Mn exposure (*42*). Also, limited information suggests the possibility that prenatal exposure of laboratory rodents to MnO_2 (via the inhalation exposure of pregnant dams) may depress neurobehavioral activity in neonatal rats, with continuing postnatal exposure of the pups possibly intensifying this depression (*85*). Thus the potential for developmental toxicity attributable to prenatal Mn exposure exists, although the minimal concentrations and durations of exposure sufficient to induce such effects are not known.

Another aspect of the composite uncertainty factor is the fact that different forms of metals may have different toxic properties. For example different oxidation states of certain metals (e.g., chromium, nickel, mercury) have different toxicities, and some researchers have suggested that different oxidation states of Mn may have quite different roles in Mn neurotoxicity (*86,87*). There are indications that the higher valence states of Mn (Mn^{+3}, Mn^{+4}) and the higher oxides in ores (Mn_2O_3 and Mn_3O_4) are more toxic (*88,89*). At present, however, insufficient information exists by which to determine the relative toxicities of different species of Mn.

Bioavailability may also figure into differences in the effects of different forms of Mn. In their 1992 report Roels et al. (*45*) noted that geometric mean blood and urinary Mn levels of workers exposed only to MnO_2 were lower than those of workers exposed to mixed oxides and salts in their 1987 report (*39*) even though airborne total dust levels were approximately the same. Mena et al. (*68*) observed no difference between the absorption of 1-μm particles of Mn chloride ($MnCl_2$) and Mn_2O_3 in healthy adults. Drown et al. (*90*) found that following intratracheal instillation of $MnCl_2$ and Mn_3O_4 in rats, the water-soluble chloride cleared four times faster than the insoluble oxide from the respiratory tract. However, despite this initial difference, after 2 weeks the amounts of labeled Mn in the respiratory tract were similar for the two compounds. Recent work by Komura and Sakamoto (*91*) comparing different forms of Mn in diet suggested that less water-soluble forms such as MnO_2 were taken up to a significantly greater degree in cerebral cortex of mice than the more soluble forms of $MnCl_2$ and Mn acetate ($Mn[CH_3COO]_2$). However, the corpus striatal binding characteristics of the +4 valence state of Mn in MnO_2 were not substantially different from those of the divalent forms in $MnCl_2$, $Mn(CH_3COO)_2$, and Mn carbonate.

Recent experimental work by Roels et al. (*92*) provides additional evidence on differences in bioavailability of different forms of Mn. Although $MnCl_2$ and MnO_2 were both bioavailable by the respiratory tract in rats, the uptake of $MnCl_2$ was significantly greater than that of MnO_2, as reflected in striatal concentrations. Moreover, MnO_2 given orally produced no significant increase in blood or cerebral tissue Mn concentrations, in contrast to approximately 70% increases over controls in rats given $MnCl_2$ by either gavage or intratracheal instillation. Once absorbed, the ability of Mn to cross the blood–brain barrier and the toxicity produced in brain regions may be a function of the oxidation state of Mn (*86,87*). In the case of Mn_3O_4, both divalent and trivalent forms of Mn are present. At this time considerable uncertainty accompanies any attempt to extrapolate from toxicity data on MnO_2 or $MnCl_2$ to the yet-to-be-determined form(s) of Mn produced by combustion of MMT in gasoline. Because insufficient data are available on the toxicologic and pharmacokinetic properties of various compounds of Mn, for the purposes of deriving an RfC for Mn no distinction was made between various compounds of Mn (*47*).

Further uncertainties come into play as the Mn RfC is used as part of the risk characterization related to MMT. Some of these issues have been intensively examined in an exchange of comments between the Ethyl Corporation and the U.S. EPA in conjunction with preparation of the MMT assessment (*93*). One rather fundamental issue concerns the relative toxicity of Mn by oral and inhalation routes. The Ethyl Corporation has questioned the biological plausibility of the RfC because it implies a roughly 100-fold difference in systemic dose from what would be associated with the oral RfD for Mn. Several points could be made about such comparisons, but a key point here is that delivered dose, not systemic dose, is critical, i.e., how much Mn actually reaches and enters a target organ such as the basal ganglia. The recent report of Roels et al. (*92*) clearly demonstrates that rats treated with either $MnCl_2$ or MnO_2 had significantly greater concentrations of Mn in the striatal region of the brain when the compounds were administered by intratracheal instillation than by gavage, despite the fact that the oral dose was 20-fold greater than the inhalation dose and produced comparable steady-state blood Mn concentrations. These findings reinforce concerns about the risks of Mn dispersed through ambient air.

Clinical and other evidence also suggests qualitative concerns that are difficult to measure quantitatively or demonstrate with currently available methods. Specifically,

much of the clinical literature on manganism refers to a psychiatric component of the illness that often involves striking emotional or mood changes that tend to appear before changes in motor function are evident. Some more recent investigations (*94*) suggest that aggressive behavior may be associated with Mn exposure (as reflected in the concentration of Mn in hair of prison inmates). However, these reports require substantiation by further studies, and the validity and relevance of hair Mn levels as an indicator of environmental Mn exposure remains to be established.

The exposure assessment was built on the PTEAM study (*62*). Given the strong design and quality assurance features of this study, it is reasonable to place a high degree of confidence in the PTEAM data as accurately representing 24-hr average Mn exposure concentrations for the Riverside population in the fall of 1990. The major uncertainties in the exposure assessment arise with the projections from these data. In essence the assessment extrapolated from PM_{10} Mn personal exposure data for a 24-hr period in the fall of 1990, when MMT was used in about 14% of gasoline at approximately 0.048 g Mn/gal, to an estimate of long-term PM_4 Mn personal exposure levels in 1995, with MMT assumed to be used in 100% of gasoline at 0.031 g Mn/gal. Several points of extrapolation are evident: particle size (PM_{10} to PM_4), which in turn was to be related to PM_5 health data; sampling period (24 hr to long-term or chronic exposure); season (no extrapolation was possible beyond the fall); past to future (1990 to 1995); extent of usage (14 to 100%); and concentration in fuel (0.048 to 0.031 g Mn/gal). Each of these extrapolations required inferences, projections, estimation procedures, or judgments. Although the intermediate steps required to reach these estimates are not described in detail here, it should be self-evident that, however reasonable and scientifically defensible these extrapolations may be, they inherently introduce uncertainties into the resulting exposure estimates. Given other approaches or assumptions, rather different projection estimates could have resulted.

Another major uncertainty for the exposure assessment concerns the relationship of these projections based specifically on Riverside, California, to other communities. The PTEAM study itself was, strictly speaking, designed to statistically represent Riverside. Nevertheless Riverside probably provides a reasonable representation of the Los Angeles basin, given the commonalities in geography, vehicle usage, and meteorology. However the relevance of the Riverside data to other U.S. communities presumably depends on similarities or differences in certain characteristics such as traffic density and meteorologic conditions. Riverside County has approximately 9 billion vehicle miles traveled (VMT) per year. Excluding the other three counties in the Los Angeles basin, about 19 other counties in the United States have higher VMT levels than Riverside. In the southwest United States, for example, Phoenix (Maricopa County), Arizona, has somewhat similar meteorologic conditions and roughly double the VMT levels of Riverside. Houston (Harris County), Texas, has about three times the VMT levels of Riverside. Notwithstanding these comparisons it remains to be determined whether the Riverside-based estimates can be applied quantitatively to any other U.S. metropolitan area. It must therefore be emphasized that the exposure assessment aspect of the MMT risk characterization is highly uncertain in its applicability to the broader U.S. population.

Research Directions

On 12 March 1991 the U.S. EPA and the National Institute of Environmental Health Sciences jointly sponsored an international symposium on health and exposure issues related to Mn and MMT, followed by a workshop on 13–15 March, which was attended by more than 100 experts in the fields of exposure and health sciences (*95*). The discussions in this workshop provided useful input to U.S. EPA scientists as they developed a program of studies that would provide information needed to support an improved, quantitative assessment of the potential risks to public health associated with MMT (*96*). Although the identification of these information needs preceded the reevaluation that figured into the 1994 U.S. EPA denial of the waiver petition by the Ethyl Corporation, the needs have remained largely unchanged since 1991.

Under health effects a major part of the rationale for the program of studies considered most needed was the premise that Mn_3O_4 was the predominant form of Mn to which human populations would be exposed in conjunction with MMT usage. The intent was to have studies performed that would serve as a basis for deriving an RfC specifically for Mn_3O_4, with the uncertainty factors reduced as much as possible to increase the precision of the RfC. The studies were to be designed to determine LOAELs and NOAELs for neurotoxicologic, reproductive, developmental, and respiratory effects of inhaled Mn_3O_4. They were to be conducted with rodents and, particularly in the case of neurotoxicologic effects, nonhuman primates. In addition the studies were intended to improve knowledge of sensitive subpopulations and provide information on Mn bioaccumulation to enable better extrapolation from subchronic to chronic effects.

Primary emphasis was placed on the use of experimental animal models rather than on human epidemiologic studies. The choice of animal models for Mn toxicity depends on the end point of interest. For respiratory effects rats (and to some extent mice) are sensitive to Mn by inhalation and other routes (*97–100*). Similarly, nonhuman primates have shown respiratory effects in relation to inhaled particulate Mn (*100*). Although reproductive effects of ingested Mn have been evaluated in rats (*101*), it appears that no rodent studies have investigated reproductive end points with inhaled Mn. However, there is no reason to believe that rodents would not serve as an adequate animal model for examining reproductive effects of Mn by inhalation. Indeed, reproductive assays using rats are specified for standard testing of F/FAs under the F/FA Rule issued by U.S. EPA (*7*).

For neurotoxicologic end points the choice of animal model is more problematic. Although rodents have shown neurochemical alterations after Mn exposure by inhalation (*85*) and noninhalation routes (*43,75–77*), they appear less likely to show behavioral effects at the same exposure levels (*102*). Primates, however, appear more sensitive to the neurotoxic effects of Mn, including disturbances of behavior and motor function (*30,32,103,104*). Some speculation has suggested that the difference in neurobehavioral sensitivity of rodents and primates may be related to the fact that, unlike primates, rodents do not have pigmented substantia nigra, which is a brain region of relatively high Mn uptake. However, it appears that other nuclei of the basal ganglia are more likely to be target sites of Mn neurotoxicity (*29*). Thus the choice of rodents or nonhuman primates for an animal model of Mn neurotoxicity should be guided in large part by the type of neurotoxicologic end point (e.g., neurochemical or neuropathologic vs neurobehavioral) to be evaluated.

Epidemiologic studies of potentially susceptible subpopulations chronically exposed predominantly to the species of Mn produced from MMT would be ideal, if feasible. However, even if such a population

could be identified (e.g., children or the elderly living in a Canadian city with substantial vehicular traffic), characterizing their inhalation and total exposure accurately would be difficult. Although group averages of urinary and blood Mn levels have been suggested to reflect very recent exposures and longer term body burden, respectively (*105*), a suitable bioindicator of Mn exposure that can differentiate inhalation exposure from oral intake does not exist (*106*). Magnetic resonance imaging and positron emission tomography techniques may offer a means of investigating the distribution of Mn *in vivo* and evaluating the integrity of function of likely targets of Mn toxicity such as the dopaminergic nigrostriatal system (*31*), but it is not clear whether these methods afford adequate resolution at levels of Mn exposure relevant to most environmental and occupational exposures levels. Although certain minimal features of study designs and end points have been specified by the U.S. EPA (*96*) (e.g., respiratory function evaluations should include flow volume, pressure volume, diffusing capacity, and ventilation and breathing mechanics), the responsibility for developing detailed protocols to achieve the objectives of these studies is expected to be borne by the Ethyl Corporation, subject to U.S. EPA approval.

A fundamental issue that affects the design of experimental health studies is the species of Mn to which human populations are exposed and, consequently, to which laboratory animals are to be exposed for testing purposes. The form or forms of particulate Mn emitted from combustion of MMT in gasoline have not been definitively established to date. U.S. EPA scientists have expressed concern that the testing protocol used to sample Mn particles in tailpipe emissions should include high enough rates and frequency of acceleration to be representative of the operation of vehicles under actual conditions. Lower rates of acceleration may not generate sufficient pressures to force some particles through the entire exhaust system and into the sample collection device. Another issue is the chemical reactions and transformations that may occur in the ambient atmosphere depending on the form of Mn emitted. The presence of acids and other compounds in the air could result in chemical changes in particulate Mn between the tailpipe where it is emitted and the nose where it is inhaled.

For improvement of the exposure assessment, the U.S. EPA has recommended obtaining personal exposure measurements using a probabilistic sampling design in areas where MMT is used in gasoline (*96*). At the time this recommendation was made, Canada was the only country where MMT was used in unleaded gasoline, and thus it was presumed that such studies would have to be conducted in Canada. However it was recognized that differences between U.S. and Canadian cities, including meteorology, traffic density, and the concentration of MMT in gasoline, would need to be taken into consideration in the design and interpretation of the studies. A stratified sampling design with particular emphasis on representation of the upper tail of the exposure distribution (e.g., through oversampling of high-exposure subgroups) was recommended. The U.S. EPA also proposed that personal exposure monitoring and ambient monitoring (preferably adjacent to each subject's residence) be conducted concurrently. Given seasonal differences in exposures, sampling during at least two different times or seasons of the year was advocated. As in the case of the health studies, it was intended that the Ethyl Corporation would take responsibility for developing detailed protocols for such exposure studies, subject to U.S. EPA approval.

Although the U.S. EPA and the Ethyl Corporation have been in communication on these proposals since 1991, the U.S. EPA has not yet formally notified Ethyl of the proposed testing requirements. Nevertheless the Ethyl Corporation funded a significant personal exposure study in Toronto, Canada, in 1995, the full results of which have not yet been made available to the U.S. EPA. Also, preliminary animal studies of Mn toxicity have been initiated by Ethyl (*74*).

The Ethyl Corporation has also indicated an intention to conduct pharmacokinetic studies of Mn by oral and inhalation routes in rodents as well as primates. In 1991 the U.S. EPA included a recommendation to characterize the bioaccumulation of inhaled Mn, primarily to support extrapolation from less-than-lifetime, high-level exposures to lifetime, low-level exposures. Although a bioaccumulation study would be especially valuable in the design and interpretation of a less-than-lifetime primate study, the U.S. EPA assigned a low priority to route-to-route pharmacokinetic studies because it was felt that such an extrapolation could introduce additional uncertainty in the derivation of an RfC. However, appropriate pharmacokinetic studies could provide useful dosimetric data for designing and interpreting toxicity studies. Such studies would need to investigate Mn disposition in blood, excreta, and target tissues such as brain regions, lung, reproductive organs, liver, and kidney. These studies should also examine Mn disposition in relation to gender and lifestage, from prenatal to senescence, and consider different oxidation states of Mn, particularly the divalent and trivalent forms.

Until the U.S. EPA formally notifies the Ethyl Corporation of the studies to be conducted under the authority of CAA Section 211(b), any of the above proposals is subject to change. Even then, as provided in the F/FA rule (*7*), the Ethyl Corporation as well as the public will be afforded an opportunity to comment on the required studies proposed by the U.S. EPA, after which the U.S. EPA may modify requirements before making them final. Although the proposed requirements are more extensive than the standard assays required under the F/FA rule, they are designed only to address specific issues directly relevant to the risk assessment of Mn in relation to MMT. In this respect some observers might prefer to characterize the prescribed studies as testing rather than research, the latter term perhaps implying a less prescriptive specification of objectives or methods. In any event it is expected that the study protocols will undergo solicited peer review to ensure that the studies will be optimally designed to address key risk assessment questions and issues. This is not to say that studies prescribed by the U.S. EPA are the only studies that could provide information of relevance to assessing the potential health risks related to MMT. A better understanding of the basic mechanisms of Mn toxicity could be quite helpful in interpreting the results of testing studies. New questions frequently arise even as testing is conducted, and independent investigations outside the focused program of studies to be prescribed by the U.S. EPA could prove valuable.

Conclusions

The importance of empirical, quantitative data on exposure and health effects for assessing risk is evident in the case of MMT. Neither assurances of benign or no detectable effects nor prophesies of impending catastrophic impacts can be given much credibility without substantially more information than has been available to date. Because MMT is already in use, the need for timely and well focused studies to better characterize the exposures and health effects resulting from this fuel additive is all the more important.

REFERENCES

1. ter Haar GL, Lenane DL, Hu JN, Brandt M. Composition, size and control of automotive exhaust particulates. J Air Pollut Control Assoc 22:39–46 (1972).
2. Piver WT. Potential dilemma: the methods of meeting automotive exhaust emission standards of the Clean Air Act of 1970. Environ Health Perspect 8:165–190 (1974).
3. U.S. Congress. Clean Air Act Amendments of 1977. PL 95-95. Washington:U.S. Government Printing Office, 1977.
4. Ethyl Corporation. MMT Q & A. Richmond, VA:Ethyl Corporation, 1997 [Online]. Available from *www.ethyl.com/mmtqa.html*
5. U.S. Environmental Protection Agency. Fuels and fuel additives; waiver decision/circuit court remand. Fed Reg 59:42227–42247 (1994).
6. Ethyl Corporation v. the U.S. Environmental Protection Agency, CA DC, 94-1505, 51 F3d, 1995.
7. U.S. Environmental Protection Agency. Fuels and fuel additives registration regulations; final rule. Fed Reg 59:33042–33142 (1994).
8. Ethyl Corporation *v.* the U.S. Environmental Protection Agency, CA DC, 94-1516, 67 F3d 941, 1995.
9. Urquhart J. Canada's House of Commons is expected to vote for ban on ethyl fuel additive. Wall Street J October 28:B6A (1996).
10. Menkes DB, Fawcett JP. Too easily lead? Health effects of gasoline additives. Environ Health Perspect 105:270–273 (1997).
11. U.S. Congress. Clean Air Act Amendments of 1990. PL 101-549. Washington:U.S. Government Printing Office, 1990.
12. Silbergeld EK. Hazards of manganese as an additive in vehicle fuel. In: International Conference: Heavy Metals in the Environment, Vol 2, September, New Orleans, LA (Lindberg SE, Hutchinson TC, eds). Edinburgh:CEP Consultants, 1987;1.
13. Davis JM, Elias RW, Grant LD. Efforts to reduce lead exposure in the United States. In: Mineral and Metal Neurotoxicology (Yasui M, Strong MJ, Ota K, Verity MA, eds). Boca Raton, FL: CRC Press, 1996;285–293.
14. Garrison AW, Cipollone MG, Wolfe NL, Swank RR Jr. Environmental fate of methylcyclopentadienyl manganese tricarbonyl. Environ Toxicol Chem 14:1859–1864 (1995).
15. U.S. Environmental Protection Agency. (1995, November). Oral reference dose (RfD) for manganese. In: IRIS (Integrated Risk Information System) [Database Online]. Available from *www.epa.gov/ngispgm3/iris/index.html*
16. Couper J. On the effects of black oxide of maganese when inhaled into the lungs. Br Ann Med Pharm 1:41–42 (1837).
17. Aschner M, Aschner JL. Manganese neurotoxicity: cellular effects and blood-brain barrier transport. Neurosci Biobehav Rev 15:333–340 (1991).
18. Murphy VA, Wadhwani KC, Smith QR, Rapoport SI. Saturable transport of manganese(II) across the rat blood-brain barrier. J Neurochem 57:948–954 (1991).
19. Rabin O, Hegedus L, Bourre J-M, Smith QR. Rapid brain uptake of manganese(II) across the blood-brain barrier. J Neurochem 61:509–517 (1993).
20. Tjälve H, Henriksson J, Tallkvist J, Larsson BS, Lindquist NG. Uptake of manganese and cadmium from the nasal mucosa into the central nervous system via olfactory pathways in rats. Pharmacol Toxicol 79:347–356 (1996).
21. ter Haar GL, Griffing ME, Brandt M, Oberding DG, Kapron M. Methylcyclopentadienyl manganese tricarbonyl as an antiknock: composition and fate of manganese exhaust products. In: 67th Annual Meeting of the Air Pollution Control Association, 9-13 June, Denver, Colorado. Pittsburgh, PA:Air Pollution Control Association, 1974.
22. National Research Council. Risk Assessment in the Federal Government: Managing the Process. Washington:National Academy Press, 1983.
23. U.S. EPA. Reevaluation of Inhalation Health Risks Associated with Methylcyclopentadienyl Manganese Tricarbonyl (MMT) in Gasoline [Final]. EPA/600/R-94/062. Washington:U.S. Environmental Protection Agency, 1994.
24. Rodier J. Manganese poisoning in Moroccan miners. Br J Ind Med 12:21–35 (1955).
25. Cotzias GC, Horiuchi K, Fuenzalida S, Mena I. Chronic manganese poisoning: clearance of tissue manganese concentrations with persistence of the neurological picture. Neurology 18:376–382 (1968).
26. Barbeau A. Etiology of Parkinson's disease: a research strategy. Can J Neurol Sci 11:24–28 (1984).
27. Wolters EC. Manganese-induced parkinsonism. In: Mineral and Metal Neurotoxicology (Yasui M, Strong MJ, Ota K, Verity MA, eds). Boca Raton, FL:CRC Press, 1996;319–325.
28. Calne DB, Chu N-S, Huang C-C, Lu C-S, Olanow W. Manganism and idiopathic parkinsonism: similarities and differences. Neurology 44:1583–1586 (1994).
29. Yamada M, Ohno S, Okayasu I, Okeda R, Hatakeyama S, Watanabe H, Ushio K, Tsukagoshi H. Chronic manganese poisoning: a neuropathological study with determination of manganese distribution in the brain. Acta Neuropathol 70:273–278 (1986).
30. Newland MC, Weiss B. Persistent effects of manganese on effortful responding and their relationship to manganese accumulation in the primate globus pallidus. Toxicol Appl Pharmacol 113:87–97 (1992).
31. Shinotoh H, Snow BJ, Hewitt KA, Pate BD, Doudet D, Nugent R, Perl DP, Olanow W, Calne DB. MRI and PET studies of manganese-intoxicated monkeys. Neurology 45:1199–1204 (1995).
32. Bird ED, Anton AH, Bullock B. The effect of manganese inhalation on basal ganglia dopamine concentrations in rhesus monkey. Neurotoxicology 5:59–65 (1984).
33. Eriksson H, Tedroff J, Thuomas K-Å, Aquilonius S-M, Hartvig P, Fasth K-J, Bjurling P, Långström B, Hedström K-G, Heilbronn E. Manganese induced brain lesions in *Macaca fascicularis* as revealed by positron emission tomography and magnetic resonance imaging. Arch Toxicol 66:403–407 (1992).
34. Heilbronn E, Eriksson H. Implications of manganese in disease, especially central nervous system disorders. In: Mineral and Metal Neurotoxicology (Yasui M, Strong MJ, Ota K, Verity MA, eds). Boca Raton, FL:CRC Press, 1996;311–317.
35. Wolters EC, Huang C-C, Clark C, Peppard RF, Okada J, Chu N-S, Adam MJ, Ruth TJ, Li D, Calne DB. Positron emission tomography in manganese intoxication. Ann Neurol 26:647–651 (1989).
36. Gavin CE, Gunter KK, Gunter TE. Mn^{2+} sequestration by mitochondria and inhibition of oxidative phosphorylation. Toxicol Appl Pharmacol 115:1–5 (1992).
37. Brouillet EP, Shinobu L, McGarvey U, Hochberg F, Beal MF. Manganese injection into the rat striatum produces excitotoxic lesions by impairing energy metabolism. Exp Neurol 120:89–94 (1993).
38. Saric M. Occupational and environmental exposures and nonspecific lung disease—a review of selected studies. Isr J Med Sci 28:509–512 (1992).
39. Roels H, Lauwerys R, Buchet J-P, Genet P, Sarhan MJ, Hanotiau I, de Fays M, Bernard A, Stanescu D. Epidemiological survey among workers exposed to manganese: effects on lung, central nervous system, and some biological indices. Am J Ind Med 11:307–327 (1987).
40. Nogawa K, Kobayashi E, Sakamoto M, Fukushima N, Ishizaki A, Makino N, Kagamimori S, Hiramaru Y, Kawano S, Kato T et al. Studies of the effects on the respiratory organs of air pollution consisting of dusts composed mainly of manganese (first report). Effects on the respiratory organs of junior high school students. Nippon Koshu Eisei Zasshi 20:315–325 (1973).

41. Cook DG, Fahn S, Brait KA. Chronic manganese intoxication. Arch Neurol 30:59–64 (1974).
42. Lauwerys R, Roels H, Genet P, Toussaint G, Bouckaert A, De Cooman S. Fertility of male workers exposed to mercury vapor or to manganese dust: a questionnaire study. Am J Ind Med 7:171–76 (1985).
43. Deskin R, Bursian SJ, Edens FW. The effect of chronic manganese administration on some neurochemical and physiological variables in neonatal rats. Gen Pharmacol 12:279–280 (1981).
44. U.S. Environmental Protection Agency. (1988, September). CRAVE cancer assessment for manganese. In: IRIS (Integrated Risk Information System) [Database Online]. Available from *www.epa.gov/ngispgm3/iris/index.html*
45. Roels HA, Ghyselen P, Buchet JP, Ceulemans E, Lauwerys RR. Assessment of the permissible exposure level to manganese in workers exposed to manganese dioxide dust. Br J Ind Med 49:25-34 (1992).
46. U.S. Environmental Protection Agency (1990, December). Inhalation reference concentration (RfC) for manganese. In: IRIS (Integrated Risk Information System) [Database Online]. Available from *www.epa.gov.ngispgm3/iris/index.html*
47. U.S. Environmental Protection Agency (1993, December). Inhalation reference concentration (RfC) for manganese. In: IRIS (Integrated Risk Information System) [Database Online]. Available from *www.epa.gov.ngispgm3/iris/index.html*
48. Mergler D, Huel G, Bowler R, Iregren A, Belanger S, Baldwin M, Tardif R, Smargiassi A, Martin L. Nervous system dysfunction among workers with long-term exposure to manganese. Environ Res 64:151–180 (1994).
49. Iregren A. Psychological test performance in foundry workers exposed to low levels of manganese. Neurotoxicol Teratol 12:673–675 (1990).
50. Lucchini R, Selis L, Folli D, Apostoli P, Mutti A, Vanoni O, Iregren A, Alessio L. Neurobehavioral effects of manganese in workers from a ferroalloy plant after temporary cessation of exposure. Scand J Work Environ Health 21:143–149 (1995).
51. American Conference of Governmental Industrial Hygienists. 1996 TLVs and BEIs: Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati, OH:American Conference of Governmental Industrial Hygienists, 1992;40–45.
52. Roels H. Attachment A-1: individual exposure and dichotomized response data of Roels (1993). In: Reevaluation of Inhalation Health Risks Associated with Methylcyclopentadienyl Manganese Tricarbonyl (MMT) in Gasoline [Final]. EPA/600/R-94/062. Washington:U.S. Environmental Protection Agency, 1994;A-66–A68.
53. Crump KS. A new method for determining allowable daily intakes. Fundam Appl Toxicol 4:854–871 (1984).
54. Hasselblad V, Jarabek AM. Dose-response analysis of toxic chemicals. In: Bayesian Biostatistics (Berry DA, Stangl DK, eds). New York:Marcel Dekker, 1994;235–259.
55. Davis JM, Jarabek A, Mage DT, Graham JA. The EPA health risk assessment of methylcyclopentadienyl manganese tricarbonyl (MMT). Risk Anal (in press).
56. Clayton CA, Perritt RL, Pellizzari ED, Thomas KW, Whitmore RW, Wallace LA, Özkaynak H, Spengler JD. Particle total exposure assessment methodology (PTEAM) study: distributions of aerosol and elemental concentrations in personal, indoor, and outdoor air samples in a southern California community. J Exposure Anal Environ Epidemiol 3:227–250 (1993).
57. Lynam DR, Pfeifer GD, ter Haar GL, Fort BF. Exposures to manganese-in-air in an urban environment. In: Trace Substances in Environmental Health: Proceedings of the 27th Annual Conference of the Society of Environmental Geochemistry and Health, July 1993, New Orleans, Louisiana (Cothern, CR, ed). Northwood, United Kingdom:Science Reviews, 1994;21–29.
58. Zayed J, Gerin M, Loranger S, Sierra P, Begin D, Kennedy G. Occupational and environmental exposure of garage workers and taxi drivers to airborne manganese arising from the use of methylcyclopentadienyl manganese tricarbonyl in unleaded gasoline. Am Ind Hyg Assoc J 55:53–58 (1994).
59. Kirshenblatt M. Unpublished data.
60. Loranger S, Zayed J, Forget E. Manganese contamination in Montreal in relation with traffic density. Water Air Soil Pollut 74:385–396 (1994).
61. Lytle CM, Smith BN, McKinnon CZ. Manganese accumulation along Utah roadways: a possible indication of motor vehicle exhaust pollution. Sci Total Environ 162:105–109 (1995).
62. Pellizzari ED, Thomas KW, Clayton CA, Whitmore RW, Shores RC, Zelon HS, Perritt RL. Particle Total Exposure Assessment Methodology (PTEAM): Riverside, California, Pilot Study. Vol I [Final Report]. EPA/600/R-93/050. Research Triangle Park, NC:U.S. Environmental Protection Agency, 1992.
63. Lönnerdal B. Dietary factors affecting trace element absorption in infants. Acta Paediatr Scand Suppl 351:109–113 (1989).
64. Fell JME, Reynolds AP, Meadows N, Khan K, Long SG, Quaghebeur G, Taylor WJ, Milla PJ. Manganese toxicity in children receiving long-term parenteral nutrition. Lancet 347:1218–1221 (1996).
65. Wilson DC, Tubman R, Bell N, Halliday HL, McMaster D. Plasma manganese, selenium and glutathione peroxidase levels in the mother and newborn infant. Early Hum Dev 26:223–226 (1991).
66. Tholin K, Sandström B, Palm R, Hallmans G. Changes in blood manganese levels during pregnancy in iron supplemented and non supplemented women. J Trace Elem Med Biol 9:13–17 (1995).
67. Kawamura R, Ikuta H, Fukuzumi S, Yamada R, Tsubaki S, Kodama T, Kurata S. Intoxication by manganese in well water. Kitasato Arch Exp Med 18:145–169 (1941).
68. Mena I, Horiuchi K, Burke K, Cotzias GC. Chronic manganese poisoning: individual susceptibility and absorption of iron. Neurology 19:1000–1006 (1969).
69. Davidsson L, Cederblad Å, Lönnerdal B, Sandström B. The effect of individual dietary components on manganese absorption in humans. Am J Clin Nutr 54:1065–1070 (1991).
70. Hauser RA, Zesiewicz TA, Martinez C, Rosemurgy AS, Olanow CW. Blood manganese correlates with brain magnetic resonance imaging changes in patients with liver disease. Can J Neurol Sci 23:95–98 (1996).
71. Krieger D, Krieger S, Jansen O, Gass P, Theilmann L, Lichtnecker H. Manganese and chronic hepatic encephalopathy. Lancet 346:270–274 (1995).
72. Davis JM, Grant LD. The sensitivity of children to lead. In: Similarities and Differences Between Children and Adults: Implications for Risk Assessment (Guzelian PS, Henry CJ, Olin SS, eds). Washington:International Life Sciences Institute Press, 1992;150–162.
73. Soliman EF, Slikker W Jr, Ali SF. Manganese-induced oxidative stress as measured by a fluorescent probe: *in vitro* study. Neurosci Res Comm 17:185–193 (1995).
74. Vitarella D, Struve MF, Goetz J, Ledford FI, Miller R, Dorman DC. Comparative neurotoxicity of oral manganese (II) chloride in neonatal and adult CD rats. In: 20th Annual Chemical Industry Institute of Toxicology Scientific Open House, 8 October, Research Triangle Park, NC. Research Triangle Park, NC:Chemical Industry Institute of Toxicology, 1996;46–47.
75. Kontur PJ, Fechter LD. Brain regional manganese levels and monoamine metabolism in manganese-treated neonatal rats. Neurotoxicol Teratol 10:295–303 (1988).
76. Seth PK, Chandra SV. Neurotransmitters and neurotransmitter receptors in developing and adult rats during manganese poisoning. Neurotoxicology 5:67–76 (1984).
77. Cotzias GC, Miller ST, Papavasiliou PS, Tang LC. Interactions between manganese and brain dopamine. Med Clin North Am 60:729–738 (1976).
78. Bell JG, Keen CL, Lönnerdal B. Higher retention of manganese in suckling than in adult rats is not due to maturational

differences in manganese uptake by rat small intestine. J Toxicol Environ Health 26:387–398 (1989).
79. Mena I. Manganese. In: Disorders of Mineral Metabolism (Bronner F, Coburn JW, eds). New York:Academic Press, 1981;233–270.
80. Dawson R Jr, Beal MF, Bondy SC, Di Monte DA, Isom GE. Excitotoxins, aging, and environmental neurotoxins: implications for understanding human neurodegenerative diseases. Toxicol Appl Pharmacol 134:1–17 (1995).
81. Desole MS, Esposito G, Migheli R, Fresu L, Sircana S, Zangani D, Miele M, Miele E. Cellular defence mechanisms in the striatum of young and aged rats subchronically exposed to manganese. Neuropharmacology 34:289–295 (1995).
82. Weiss B, Reuhl K. Delayed neurotoxicity: a silent toxicity. In: Neurological Disease and Therapy. Vol 26: Principles of Neurotoxicology (Chang LW, ed). New York:Marcel Dekker, 1994;765–784.
83. Wennberg A. Neurotoxic effects of selected metals. Scand J Work Environ Health 20(Special Issue):65–71 (1994).
84. Smyth LT, Ruhf RC, Whitman NE, Dugan T. Clinical manganism and exposure to manganese in the production and processing of ferromanganese alloy. J Occup Med 15:101–109 (1973).
85. Lown BA, Morganti JB, D'Agostino R, Stineman CH, Massaro EJ. Effects on the postnatal development of the mouse of preconception, postconception and/or suckling exposure to manganese via maternal inhalation exposure to MnO_2 dust. Neurotoxicology 5:119–131 (1984).
86. Archibald FS, Tyree C. Manganese poisoning and the attack of trivalent manganese upon catecholamines. Arch Biochem Biophys 256:638–650 (1987).
87. Donaldson J, McGregor D, LaBella F. Manganese neurotoxicity: a model for free radical mediated neurodegeneration? Can J Physiol Pharmacol 60:1398–1405 (1982).
88. Ali SF, Duhart HM, Newport GD, Lipe GW, Slikker W Jr. Manganese-induced reactive oxygen species: comparison between Mn^{+2} and Mn^{+3}. Neurodegeneration 4:329–334 (1995).
89. Oberdoerster G, Cherian G. Manganese. In: Biological Monitoring of Toxic Metals (Clarkson TW, Friber L, Nordberg GF, Sager PR, eds). New York:Plenum Press, 1988;283–301.
90. Drown DB, Oberg SG, Sharma RP. Pulmonary clearance of soluble and insoluble forms of manganese. J Toxicol Environ Health 17:201–212 (1986).
91. Komura J, Sakamoto M. Subcellular and gel chromatographic distribution of manganese in the mouse brain: relation to the chemical form of chronically-ingested manganese. Toxicol Lett 66:287–294 (1993).
92. Roels H, Meiers G, Delos M, Ortega I, Lauwerys R, Buchet JP, Lison D. Influence of the route of administration and the chemical form ($MnCl_2$, MnO_2) on the absorption and cerebral distribution of manganese in rats. Arch Toxicol 71:223–230 (1997).
93. U.S. EPA. ORD's Response to Public Comments on Health and Exposure Issues in Relation to the Waiver Petition of Ethyl Corporation to Add Methylcyclopentadienyl Manganese Tricarbonyl (MMT) to Unleaded Gasoline. Washington:U.S. Environmental Protection Agency, 1994.
94. Gottschalk LA, Rebello T, Buchsbaum MS, Tucker HG, Hodges EL. Abnormalities in hair trace elements as indicators of aberrant behavior. Compr Psychiatry 32:229–237 (1991).
95. U.S. EPA. Summary of Workshop Discussions at the Manganese and Methylcyclopentadienyl Manganese Tricarbonyl (MMT) Conference, 12–15 March, Washington. Washington:U.S. Environmental Protection Agency, 1991.
96. U.S. EPA. Information Needed to Improve the Risk Characterization of Manganese Tetraoxide (Mn_3O_4) and Methylcyclopentadienyl Manganese Tricarbonyl (MMT). Washington:U.S. Environmental Protection Agency, 1991.
97. Shiotsuka RN. Inhalation toxicity of manganese dioxide and a magnesium oxide-manganese dioxide mixture. Army Project Order No 3803. Frederick, MD:U.S. Army Medical Research and Development Command, 1984.
98. Moore W, Hysell D, Miller R, Malanchuk M, Hinners R, Yang Y, Stara JF. Exposure of laboratory animals to atmospheric manganese from automotive emissions. Environ Res 9:274–284 (1975).
99. Lloyd Davies TA, Harding HE. Manganese pneumonitis: further clinical and experimental observations. Br J Ind Med 6:82–90 (1949).
100. Nishiyama K, Suzuki Y, Fujii N, Yano H, Miyai T, Ohmishi K. Effect of long-term inhalation of manganese dusts. II: Continuous observation of the respiratory organs of monkeys and mice. Nihon Eiseigaku Zasshi 30:117 (1975).
101. Laskey JW, Rehnberg GL, Hein JF, Carter SD. Effects of chronic manganese (Mn_3O_4) exposure on selected reproductive parameters in rats. J Toxicol Environ Health 9:677–687 (1982).
102. Morganti JB, Lown BA, Stineman CH, D'Agostino RB, Massaro EJ. Uptake, distribution and behavioral effects of inhalation exposure to manganese (MnO_2) in the adult mouse. Neurotoxicology 6:1–16 (1985).
103. Suzuki Y, Fujii N, Yano H, Ohkita T, Ichikawa A, Nishiyama K. Effects of the inhalation of manganese dioxide dust on monkey lungs. Tokushima J Exp Med 25:119–125.
104. Eriksson H, Mägiste K, Plantin L-O, Fonnum F, Hedström K-G, Theodorsson-Norheim E, Kristensson K, Stalberg E, Heilbronn E. Effects of manganese oxide on monkeys as revealed by a combined neurochemical, histological and neurophysiological evaluation. Arch Toxicol 61:46–52 (1987).
105. Roels H, Lauwerys R, Genet P, Sarhan MJ, de Fays M, Hanotiau I, Buchet J-P. Relationship between external and internal parameters of exposure to manganese in workers from a manganese oxide and salt producing plant. Am J Ind Med 11:297–305 (1987).
106. Gerhardsson L, Skerfving S. Concepts on biological markers and biomonitoring for metal toxicity. In: Toxicology of Metals (Chang LW, Magos L, Suzuki T, eds). Boca Raton, FL:CRC Lewis, 1996;81–107.